MICROHABITAT PREFERENCES BY AQUATIC INVERTEBRATES INFLUENCE BIOASSESSMENT METRICS IN PIEDMONT STREAMS OF GEORGIA AND ALABAMA

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Abstract. The U.S. Geological Survey analyzed macroinvertebrate samples from woody debris and riffle habitats in 10 small Piedmont streams in Georgia and Alabama to determine if habitat preferences influence commonly used invertebrate community metrics. Eighty-seven commonly used metrics were compared, and 11 (13 percent) were found to be significantly different between habitats. Woody debris habitat had slightly higher taxa richness, whereas riffles had higher overall abundances and densities. Abundance metrics that differed significantly were Ephemeroptera, Plecoptera, and Tricoptera (EPT), Tricoptera, *Corbicula*, collector-gatherer, filtering-collector, and total abundance. Richness metrics that were significantly different were midge, Diptera, omnivore, and shredder. *Corbicula* richness (one species) was the only richness metric that scored higher in riffle habitats. These results indicate that Piedmont biomonitoring studies that do not sample riffle habitat may underestimate sensitive EPT taxa because of a lack of habitat availability rather than changes in water-quality conditions. Furthermore, this study indicates the possible need for a correction factor to be applied to ecological condition metrics used by the State of Georgia that adjusts for the presence or absence of riffle habitat in Piedmont streams.

INTRODUCTION

The increased use of aquatic invertebrate community data in biomonitoring and environmental compliance studies underscores the need for a better understanding of the relation between microhabitat preferences by invertebrates and how these preferences might affect commonly used metrics and/or biotic indices. The potential for sampling bias is especially relevant when protocols recommend targeting a specific microhabitat or if sampled reaches lack specific microhabitats. Microhabitat comparability is also important when analyzing invertebrate data for evidence of episodic disturbances or general declines in water quality across multiple streams.

Published protocols commonly prescribe sampling invertebrates from either woody debris or riffle habitat as part of their standard operating procures. For example, the Georgia Environmental Protection Division (GaEPD) prescribes qualitative sampling of all available microhabitats such as riffles, woody debris/snags, sand, undercut bank/root mats, and leaf packs in Piedmont streams (Georgia Environmental Protection Division, 2002). Similarly, the U.S. Environmental Protection Agency's (USEPA) Environmental Monitoring and Assessment Program (EMAP) follows the protocol of Barbour and others (1999), which prescribes the qualitative sampling of multiple habitats including riffles, woody debris, undercut banks, leaf packs, and pools. The U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Program protocol prescribes quantitative sampling of specifically targeted, highly productive microhabitat, either riffles or woody debris, as well as the additional collection of a qualitative multihabitat sample (Moulton and others, 2002). The general assumption in the NAWQA protocols is that riffle habitat will always be present in Piedmont stream reaches and that woody debris will be sampled in lower gradient streams such as those in the Coastal Plain. During 2002, however, reconnaissance surveys of 150 Piedmont streams indicated anecdotally that riffle habitat was present in only approximately one-third of small (20–50 mi^2 drainages), wadeable, and publicly accessible stream reaches and that riffle habitat was more commonly present in Upper Piedmont rather than Lower Piedmont streams. Obviously, the presence or absence of riffle habitat is primarily due to local physical controls on a stream, such as streambed gradient and upstream channel conditions (Thorne, 1997); however, these factors are usually not considered when deciding where to sample for local watershed assessments. Furthermore, probabilistic sampling designs, by nature, fail to consider the comparability of microhabitat in reaches that are chosen in a purely random manner even though the comparability of habitats among streams may be critical to determining the validity of invertebrate monitoring data collected for scientific studies as well as for other regulatory purposes.

The purpose of this study is to determine major differences, if any, between invertebrate communities inhabiting riffle and woody debris microhabitats in small, wadeable Piedmont streams and to determine whether sampling one microhabitat over another might significantly affect the results of the application of the invertebrate community data to commonly used metrics. The hypothesis tested is that some differences in invertebrate communities may be apparent at the species level owing to habitat preferences by individual taxa, but that invertebrate community metrics would not be significantly different due to exclusively sampling either riffle or woody debris habitat in the same stream.

METHODS

Ten Piedmont streams were selected from watersheds in Georgia and Alabama and were sampled during spring 2003 as part of a larger USGS NAWQA urban land-use study, which included sampling ecological, water-quality, and hydrologic conditions at 30 sites characterized by a range of urban development (Gregory and Bryant, 2003; Table 1 and Fig. 1). At all of these sites invertebrates were collected according to the NAWQA protocol (Moulton and others, 2002), which included a qualitative multihabitat sample as well as a quantitative sample from both woody debris and riffles. Data from the qualitative samples were not analyzed as part of this report.

Woody debris samples were collected from stable, submerged pieces of wood that had been submerged long enough to be colonized by invertebrates and algae. If these pieces of woody debris were too large to move or cut, they were processed in place by placing a D-fame net (500 micron mesh) downstream of the piece of wood and vigorously sweeping the epilithic material into the net with a brush. Smaller pieces of wood were removed and processed in a 5-gallon bucket using clean water (sieved through 500 micron mesh) to wash epilithic organisms from the collected woody debris. These pieces were visually inspected after 10–30 minutes to collect any additional organisms that emerged from holes and crevices. Materials from at least 10 pieces of wood were composited into a single sample from the site. Depth of water, depth of woody debris, water velocity, and bottom substrate composition were measured at each sampling location, and sampled areas from all pieces of wood were estimated using calipers and recorded on data sheets.

Riffles were sampled with a modified surber sampler (500 micron mesh) equipped with a 50-centimeters (cm) x 50-cm standardization frame. The net was placed in the area to be sampled and the material just upstream of the net and inside the standardization frame was disturbed so that epilithic organisms were dislodged and moved into the net. Additionally, each piece of cobble was individually removed and brushed into the net to ensure that organisms not easily removed also would be collected. Samples were collected at a minimum of five different locations within the reach. If more than one riffle was present, samples were spatially distributed to be representative of the entire reach. Depth, velocity, substrate composition, and embeddedness were measured at each sampling location with the surber sampler and recorded on data sheets.

Table 1. U.S. Geological Survey station identification numbers, station names, watershed areas, and urban index values[1] for sites sampled in the upper and lower Piedmont Ecoregion of Georgia and Alabama.

[ID, identification; USGS, U.S. Geological Survey; bold sites are part of National Water-Quality Assessment trend network]

Map ID (Figure 1)	USGS station ID	Station name	Watershed area (square miles)	Urban index
1	02338523	**Hillabahatchee Creek at Thaxton Road near Franklin, Georgia**	17	5
2	02347748	Auchumpkee Creek at Allen Road near Roberta, Georgia	43	9
3	02338375	Centralhatchee Creek near Centralhatchee, Georgia	34	11
4	02221000	Murder Creek near Monticello, Georgia	24	14
5	02338280	Whooping Creek at State Route 5 near Whitesburg, Georgia	27	18
6	02337395	Dog River at North Helton Road near Winston, Georgia	44	30
7	02204468	Walnut Creek at Airline Road near McDonough, Georgia	49	41
8	02336728	Utoy Creek at Great Soutwest Parkway near Atlanta, Georgia	34	73
9	02335870	**Sope Creek near Marietta, Georgia**	31	91
10	02335910	Rottenwood Creek at Interstate Parkway near Smyrna, Georgia	19	96

[1]Urban index values refers to the urban character of each basin and was calculated using factors such as land use, infrastructure, population, socioeconomic characteristics, and estimates of impervious surface (McMahon and Cuffney, 2000).

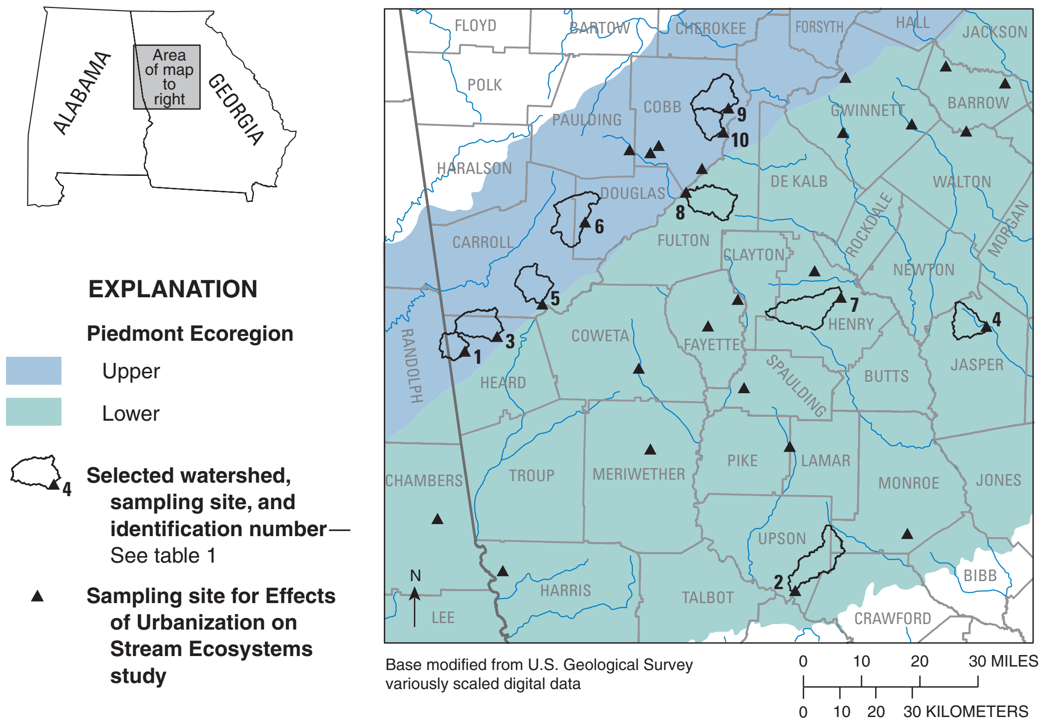

Figure 1. Watersheds sampled as part of the effects of urbanization on stream ecosystems study in the Piedmont ecoregion in the vicinity of Metropolitan Atlanta, Georgia.

All invertebrate samples were preserved in the field and shipped to the USGS National Water-Quality Laboratory in Denver, Colorado, where the Biological Group processed samples and provided invertebrate taxonomic and abundance data using standard NAWQA Program methods (Moulton and others, 2000). Taxonomic ambiguities in the invertebrate data sets were resolved using the Invertebrate Data Analysis System (IDAS) software (Version 3.1) (Cuffney, 2003). Eighty-seven commonly used community metrics, tolerance values, and similarity and diversity index values were calculated using IDAS. The untransformed metric values calculated by using IDAS from the communities found on the woody debris and riffle habitats were statistically analyzed and graphed using S-plus software (Insightful Corp.[1], 2002). Between-group comparisons of each metric was made using the Wilcoxon Rank-Sum Test (Wilcoxon, 1945), a nonparametric alternative to the two-sample *t*-test.

RESULTS

In general, sampled pieces of woody debris were located in deeper, slower-moving water and over slightly smaller substrates than riffle habitat, whereas sampled riffle areas were located in shallower areas with somewhat higher current velocities (Table 2). Riffle microhabitat sampled also tended to be composed of somewhat embedded pieces of small (64–128 mm) cobble. Sampled surface areas were larger on average and more variable for sampling the woody debris than for sampling the riffle areas (Table 2). The total sampling area for each riffle sample was 12,500 cm^2, whereas the area of woody debris habitat sampled ranged from 11,000 to 31,100 cm^2. The average size of pieces of woody debris sampled was 11.6 cm diameter by 47 cm long.

Table 2. Microhabitat characteristics of riffle and woody debris across all sites.

[m, meter; m/second, meter per second; cm, centimeter; cm^2, square centimeter; #/m^2, number per square meter; NA, not applicable]

Microhabitat characteristic	Riffle (n = 45)	Woody debris (n = 102)
Depth (m)	0.16	0.30
Velocity (m/second)	0.49	0.29
Snag depth (cm)	NA	9
Snag diameter (cm)	NA	11.6
Snag length (cm)	NA	47
Dominant benthic substrate	small cobble	very coarse gravel
Embeddedness (percent)	31	NA
Average area sampled (cm^2)	[1]12,500	20,300
Range of sampled areas (cm^2)	NA	11,100–32,000
Mean density of individuals (#/m^2)	8.11	4.42

[1]All samples in riffle habitat sampled the same area.

[1]Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

A total of 29,765 individuals were collected from the 10 paired samples. Overall, invertebrates on riffle habitat were more abundant with 16,170 individuals collected compared with 13,595 individuals collected from woody debris habitat. Across all sites, woody debris habitat was slightly more diverse, with a total of 162 taxa compared with 156 taxa for riffles. Riffle habitat also had higher maximum mean density with 116 individuals per square meter (ind m^{-2}), whereas woody debris maximum mean density was only 107 ind m^{-2}. Average mean densities of invertebrates were almost twice as high in riffle habitat (8.1 ind m^{-2}) as on woody debris (4.4 ind m^{-2}).

Twenty-five of the 200 species collected exhibited a preference for either wood or riffle habitat exclusively; 15 taxa were collected only on wood compared with 10 taxa that were collected only in riffles (Table 3). The woody debris habitat specialists were dominated (9 taxa) by the Chironomidae family (Order: Diptera), whereas riffle specialists were a diverse group with taxa from several orders including Megaloptera, Coleoptera, Ephemeroptera, Annelida, Odonata, and Lumbriculida (Table 3). Only two microhabitat specialists, *Orthocladius lignicola* (woody debris) and *Corydalus cornutus* (riffle), were collected from at least 50% of the sites.

Table 3. Names of species and mean densities found exclusively on either woody debris or in riffle habitat in Piedmont streams.

[m^2, square meter]

Family	Scientific name	Woody debris	Riffles	Mean density (number/m^2)
Chironomidae	*Orthocladius lignicola (Kieffer)*	5	0	1.23
Do.	*Ablabesmyia sp.*	3	0	0.61
Do.	Chironomini	3	0	0.77
Do.	*Micropsectra/Tanytarsus sp.*	3	0	0.53
Perlodidae	*Isoperla sp.*	3	0	1.60
Veliidae	*Microvelia sp.*	3	0	0.81
Aeshnidae	*Boyeria vinosa (Say)*	2	0	0.12
Chironomidae	Diamesinae	2	0	0.98
Do.	*Pagastiella sp.*	2	0	0.29
Do.	*Paratendipes sp.*	2	0	0.66
Do.	*Procladius sp.*	2	0	0.14
Do.	*Tribelos sp.*	2	0	0.19
Peltoperlidae	Peltoperlidae	2	0	1.08
Polycentropodidae	*Paranyctiophylax sp.*	2	0	0.28
Staphylinidae	Staphylinidae	2	0	1.11
Corydalidae	*Corydalus cornutus (Linnaeus)*	0	5	1.6
Elmidae	*Optioservus ovalis (LeConte)*	0	3	1.6
Heptageniidae	*Leucrocuta sp.*	0	3	2
Perlidae	*Neoperla sp.*	0	3	2.88
Lumbriculidae	Lumbriculidae	0	2	4.8
*	Annelida	0	2	0.16
Chironomidae	*Micropsectra sp.*	0	2	0.56
Coenagrionidae	*Argia sp.*	0	2	1.76
Corydalidae	*Nigronia serricornis (Say)*	0	2	0.16
Elmidae	*Promoresia sp.*	0	2	0.8

*This organism was not identified below the family level.

Although several invertebrate species tended to prefer one microhabitat over the other, only 11 of the calculated metrics (13%) showed significant differences between woody debris and riffle habitat ($\alpha \leq 0.1$). Of these, abundance metrics tended to be the most sensitive to the type of habitat sampled, with four metrics showing significant differences. Three richness metrics showed significant differences between the two habitat types, whereas only two functional group richness metrics and two functional group abundance metrics exhibited significant differences between wood and riffle habitats (Table 4).

Of all the metrics that showed significant differences, the highest levels of significance ($p \leq 0.01$) were exhibited by richness composed of midges, richness composed of Diptera, and richness composed of omnivores (Figs. 2 and 3). Less significantly different ($0.01 < p \leq 0.05$) were EPT abundance, Tricopteran abundance, and collector-gatherer abundance (Fig. 2). Least significantly different ($0.05 \leq p < 0.1$) were *Corbicula* abundance, filter-collector abundance, richness composed of *Corbicula*, and richness composed of shredders (Figs. 2 and 3).

Of the 11 metrics that were significantly different, median metric values were higher on invertebrate data calculated from communities found on riffle habitat in 7 instances, whereas metric values were higher on data collected from wood in only 4 instances. Metrics that exhibited higher values on riffle habitat were total abundance, EPT abundance, Tricoptera abundance, *Corbicula* abundance collector-gatherer abundance, filtering collector abundance, and richness composed of *Corbicula* (Figs. 2 and 3). Metrics that exhibited higher values on woody debris included richness composed of midges, Diptera, omnivores, and shredders (Fig. 3).

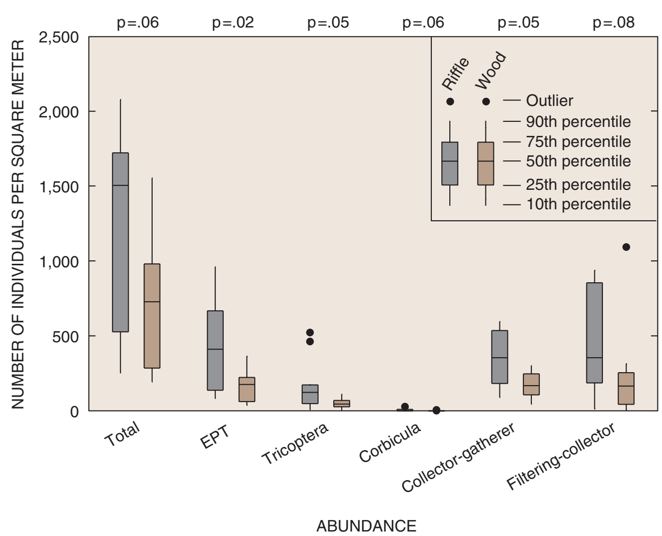

Figure 2. Differences between abundance metrics on riffle and wood habitats. [p = p-value, attained significance level of data; EPT, Ephemeroptera, Plecoptera, and Tricoptera]

Table 4. Metrics calculated from invertebrate data collected on woody debris and riffle habitats from sites sampled during 2003.

[Shaded metrics denote significantly different communities in woody debris and riffle habitats (differences, p< 0.1); EPA, Environmental Protection Agency]

Richness metrics
Total richness (number of nonambiguous taxa)
Richness composed of mayflies, stoneflies, and caddisflies (EPT)
Ratio of EPT richness to midge richness
Richness composed of mayflies
Richness composed of stoneflies
Richness composed of Pteronarcys
Richness composed of caddisflies
Richness composed of odonates
Richness composed of Coleoptera
Richness composed of Diptera
Richness composed of midges
Richness composed of Orthocladinae midges
Ratio of orthoclad richness to midge richness
Richness composed of Tanytarsanii midges
Ratio of Tanytarsanii richness to midge richness
Richness composed of non-midge Diptera
Richness composed of non-insects
Richness composed of nonmidge Diptera and non-insects
Richness composed of molluscs and crustaceans
Richness composed of Gastropoda
Richness composed of Bivalvia
Richness composed of Cobricula
Richness composed of Amphipoda
Richness composed of Isopoda
Richness composed of Oligochaeta
Abundance metrics
Total number of organisms in the sample
Abundance of EPT (Ephemeroptera, Plecoptera, Trichoptera)
Ratio of EPT abundance to midge abundance
Abundance of mayflies
Abundance of stoneflies
Abundance of Pteronarcys
Abundance of caddisflies
Abundance of Odonata
Abundance of Coleoptera
Abundance of Diptera
Abundance of midges
Abundance of Orthocladinae midges
Abundance of Tanytarsanii midges
Abundance of non-midge Diptera
Abundance of non-insects
Percentage of abundance composed of nonmidge Diptera and noninsects
Abundance of Mollusca and Crustacea
Abundance of Gastropoda
Abundance of Bivalvia
Abundance of Corbicula
Abundance of Amphipoda
Abundance of Isopoda
Functional group richness metrics
Richness composed of parasites
Richness composed of predators
Richness composed of omnivores
Richness composed of collector-gatherers
Richness composed of filtering-collectors
Richness composed of scrapers
Richness composed of shredders
Richness composed of piercers
Functional group abundance metrics
Abundance composed of parasites
Abundance composed of predators
Abundance composed of omnivores
Abundance composed of collector-gatherers
Abundance composed of filtering-collectors
Abundance composed of scrapers
Abundance composed of shredders
Abundance composed of piercers
Tolerance metrics
Average EPA tolerance values for sample based on richness
Abundance-weighted EPA tolerance value for sample
Percentage abundance of dominant taxa
Percentage of total abundance represented by the most abundant taxon
Percentage of total abundance represented by the two most abundant taxon
Percentage of total abundance represented by the three most abundant taxon
Percentage of total abundance represented by the four most abundant taxon
Percentage of total abundance represented by the five most abundant taxon
Diversity metrics
Margalef's diversity
Menhinick's diversity
Simpson's dominance
Simpson's diversity
Brillouin's diversity
Shannon's diversity
Simpson's evenness
Brillouin's evenness
Shannon's evenness
Similarity indices
Jaccard coefficient
Sørenson coefficient
Proportional similarity
Euclidean distance
Morisita's index
Horn's index
Bray-Curtis dissimilarity
Pinkham and Pearson's index

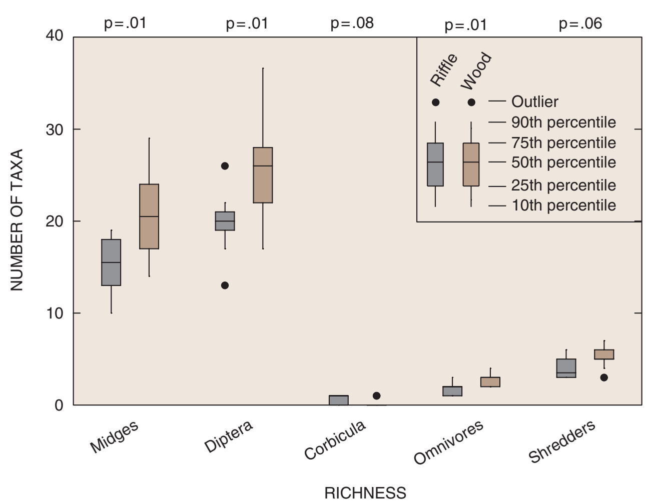

Figure 3. Differences between richness metrics on riffle and wood habitats. [p = p-value, attained significance level of data]

DISCUSSION

Of the 33 richness metrics compared in this study, only 5—richness of Diptera, midges, *Corbicula*, omnivores, and shredders—were significantly different, with greater numbers of taxa inhabiting woody debris rather than riffle habitat. Most of the differences in terms of richness, however, can be attributed to differences in a single group of aquatic insects, the midges. Furthermore, four of the richness metrics are codependent since midges are a family within the Dipteran order and most of the midges are classified functionally as either shredders or omnivores. *Corbicula* "richness" was also significantly different and higher in riffles; however, this metric may not actually provide any useful information in terms of the overall invertebrate community since all *Corbicula* collected in this study were of a single species, *Corbicula fluminea*. Therefore, this metric functions as a binary response variable, indicating that *Corbicula fluminea* are not usually found on woody debris.

Perhaps the most significant differences between the woody debris and riffle communities were in terms of abundance metrics. Of the 30 abundance metrics compared, 6 were significantly different between woody debris and riffle habitats. Total abundance, abundance of EPT taxa, caddisflies, *Corbicula*, collector-gatherers, and filtering-collectors were all significantly higher in riffle habitat rather than on woody debris (Fig. 2). Like the corelated richness groups, the multiple differences among the abundance metrics are due mainly to a single group of invertebrates. In the case of riffle habitat, Tricopteran taxa are the main group responsible for differences in EPT abundance and may also be responsible for differences in total abundance since some species of Tricoptera are commonly abundant in riffle habitat, especially in more urbanized streams. *Cheumatopsyche* sp., for example, was found in densities as great as 288 ind m^{-2} during this study.

Even though other environmental factors beyond the scope of this study may also influence the response of the invertebrate communities, the results of this study show that the assumption that riffle habitat is the richest habitat for sampling invertebrate communities may be in error. When samples are analyzed with a high degree of taxonomic resolution, the number of taxa in difficult-to-identify groups such Diptera may result in woody debris habitat actually having higher overall species richness. Abundance, however, tended to be higher in riffle habitat, especially in terms of species or metrics that are considered sensitive, such as EPT taxa. Differences in functional group metrics reflect these major differences in abundance and richness of major groups that were significantly different such as midges and Tricopteran taxa. Biological assessment protocols that use EPT index, abundance of collector gatherers, or abundance of filtering-collectors as metrics for the comparison of Piedmont streams may benefit from an adjustment factor based on the presence or absence of riffle habitat to accurately reflect differences that are due to differences in water quality and not due to habitat availability.

LITERATURE CITED

Barbour, M.T., Jeroen Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish. Second Ed. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C., 339 pp.

Cuffney, T.F. 2003. User's manual for the National Water-Quality Assessment Program invertebrate data analysis system (IDAS) software: Version 3. U.S. Geological Survey Open-File Report 03-172, 103 pp.

Gregory, M.B., and W.L. Bryant. 2003. Effects of urbanization on stream ecosystems in the piedmont ecoregion of Georgia and Alabama—a study design. In *Proceedings of the 2003 Georgia Water Resources Conference*, April 23–24, 2003, at The University of Georgia. Kathryn J. Hatcher (ed.), Institute of Ecology, The University of Georgia, Athens, Georgia, CD–ROM.

Georgia Environmental Protection Division. 2002. Draft standard operating procedures: freshwater macroinvertebrate biological assessment: Atlanta, Ga. Georgia Department of Natural Resources, Water Protection Branch, 102 pp.

Insightful Corporation. 2002. S-Plus Professional Edition version 6.1, Copyright 2002. Lucent Technologies, Inc.

McMahon, Gerard, and T.F. Cuffney. 2000. Quantifying urban intensity in drainage basins for assessing stream ecological conditions. *Journal of the American Water Resources Association*, v. 36, no. 6, pp. 1247–1261.

Moulton, S.R., II, J.L. Carter, S.A. Grotheer, T.F. Cuffney, and T.M. Short. 2000. Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory—Processing, taxonomy, and quality control of benthic macroinvertebrate samples. U.S. Geological Survey Open-File Report 00-212, 49 pp.

Moulton, S.R., II, J.G. Kennen, R.M. Goldstein, and J.A. Hambrook. 2002. Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program. U.S. Geological Survey Open-File Report 02-150, 87 pp. Accessed January 13, 2005, at *http://water.usgs.gov/nawqa/protocols/OFR02-150/OFR02-150.pdf*

Thorne, C.R. 1997. Channel types and morphological classification, In *Applied Fluvial Geomorphology for River Engineering and Management*. C.R. Thorne, R.D. Hey and M.D. Newson (eds.), John Wiley & Sons Ltd., pp. 175–222.

Wilcoxon, F.A. 1945. Individual comparisons by ranking methods, *Biometrics*, v. 1, pp. 80–83.